

# An Update on Antibody-Mediated Diseases



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It doesn't seem that long since 2001 when I summarised the diseases of the neuromuscular junction caused by autoantibodies to ion channels (VGCC, VGKC) and receptors (AChR, MuSK) but there are, nevertheless, a surprising number of developments that you might like to hear about in this brief review. The table has been updated to include all of the known conditions in which antibodies – if not yet shown formally to be the pathogenic mechanism – are proving crucial to the diagnosis and thence the treatment of patients.

### What's been happening at the neuromuscular junction?

Perhaps one of the most important aspects for the general neurologist is the surprising frequency of AChR-Ab positive MG in the elderly a few of whom may have been previously unrecognized, or misdiagnosed as motor neuron disease or stroke.<sup>1</sup> The changing demographics probably reflect better diagnosis, greater awareness and ascertainment, and the increasing age of the population, but there may also be a real increase in the disease in older people.

With the advent of a relatively easy diagnostic testing for MuSK antibodies there have been many studies on MuSK-MG from around the world indicating that it is recognised widely, although the prevalence appears to vary considerably, probably reflecting environmental factors, with the highest prevalence in countries in Southern Europe and the equivalent USA states – the prevalence decreases in countries further north. Whereas the pathogenic mechanisms in AChR-Ab positive MG (AChR-MG) are well established, the mechanisms in MuSK-MG are much less clear. The antibodies bind to the extracellular domains of MuSK and affect remodelling of the neuromuscular junction in various active and passive immunisation models but AChR numbers are not greatly altered in patients and these changes alone would not adequately account for the marked defect in neuromuscular transmission in MG. Moreover, the MuSK-MG patients often have particularly severe bulbar involvement which suggests that these muscles, which are often atrophied, may be particularly susceptible to the effects of the antibodies; this requires further study.<sup>2</sup> Importantly, the thymus is usually normal in MuSK-MG and thymectomy not (in our view) indicated, and although plasma exchange is always effective, MuSK-MG can be relatively difficult to treat; studies on the effects of newer immunotherapies such as rituximab are begin-

ning to appear and may offer hope to those patients with intractable MuSK-MG.<sup>3</sup>

The patients who have neither AChR nor MuSK antibodies by current techniques are still somewhat of a mystery, but there are some developments. For instance, using AChRs expressed in a human kidney cell line (HEK 293 cells), and clustered into dense arrays similar to those on the postsynaptic membrane of the NMJ, we found binding of antibodies in patients previously negative for AChR antibodies, and a similar method has also improved the detection of MuSK antibodies; unfortunately these techniques are not yet adapted for routine diagnostics.<sup>4</sup> By contrast, there have been few developments in LEMS or in acquired neuromyotonia although a number of clinical studies from the Netherlands have detailed the epidemiology, cancer incidence and risk factors in LEMS.<sup>5</sup>

### Moving to the central nervous system

Previous generations seemed to have had quite fixed ideas regarding antibodies and CNS disease – understandably drawing attention to the “blood brain barrier” as a reason why antibodies would not get into the parenchyma of the CNS. Nevertheless, there is always some IgG in the cerebrospinal fluid (approximately 1/400 of the serum levels) and quantitative testing can demonstrate the relationship between serum and CSF levels of specific autoantibodies – intrathecal synthesis of specific autoantibodies may be detectable even when the intrathecal concentration of total IgG is within normal limits. However, in my view, we shouldn't necessarily assume that intrathecal synthesis of a particular antibody is required for it to be pathogenic – it is possible that some diseases are caused by local leakage of antibody from the serum into the brain parenchyma. But this view is not shared by many and it's an area for future study!

In 2001 we first showed that VGKC antibodies were associated not only with neuromyotonia but also with both limbic encephalitis and Morvan's syndrome. A patient with marked neuromyotonia, autonomic, sleep disturbance and cognitive dysfunction (Morvan's) had high VGKC antibodies and improved after multiple plasma exchanges, whereas limbic encephalitis that responded to plasma exchange was seen in two women, one with thymoma and MG, and the other with no tumour. Slowly these cases began to be recognised and described and VGKC antibody testing has now become routine, at least in some countries, for patients with unexplained memory loss, seizures and personality/psychiatric

**Table: Update on antibody targets and associated conditions**

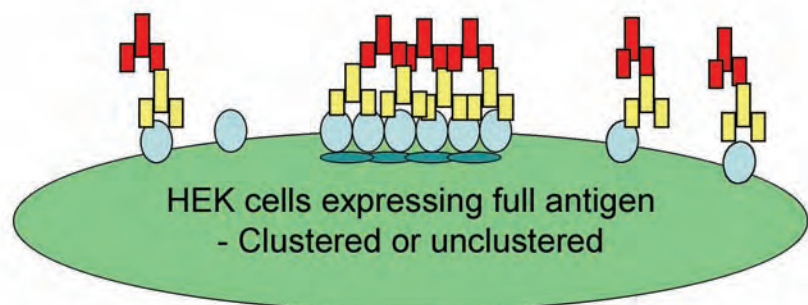
TARGET	ANTIGEN USED FOR ASSAY	CONDITIONS	NEW DEVELOPMENTS SINCE 2001
AChR	125I-bungarotoxin-AChR (fetal and adult)	Myasthenia gravis Arthrogryposis multiplex congenital	Cell-based assay using clustered AChR to increase sensitivity
MuSK	Recombinant extracellular domain of MuSK on ELISA	Seronegative (or MuSK antibody positive) myasthenia gravis	Commercially available radioimmunoprecipitation assay and cell-based MuSK expression to increase sensitivity
VGCC	125I-conotoxin-MV1IC VGCC	Lambert Eaton myasthenic syndrome with or without small cell lung cancer	
VGKC	125I-dendrotoxin-VGKC	Acquired neuromyotonia Limbic and hypothalamic syndromes with or without neuromyotonia	
NEW TARGETS	ASSAY METHOD	CONDITION	TREATMENT EFFECT
VGKC	125I-dendrotoxin-VGKC but cell-based methods will be developed for new antigens	Increasing recognition of "VGKC" antibodies in limbic or epilepsy syndromes and in Morvan's syndrome.	Usually very immunotherapy-responsive. There can also be frequent brief partial seizures. <sup>18</sup>
Aquaporin 4	Various including NMO-IgG and cell-based, ELISAs	Distinguishes neuromyelitis optica from multiple sclerosis or other causes of optic neuritis or transverse myelitis	Requires aggressive immunotherapies to treat and prevent relapses
NMDAR	Cell based expression of NR1/NR2B	A newly-described syndrome including both limbic and subcortical features with prominent movement disorders often in young adults and children. Often associated with ovarian teratoma but can be non-paraneoplastic.	Either by tumour removal, and/or by immunotherapies.
AMPA or GABA(B)R	Cell based expression of GluR2/3 Cell based expression of GABA(B)R	Limbic encephalitis often associated with tumours Limbic encephalitis often associated with tumours	Immunotherapy responsive but can relapse Immunotherapy responsive
GlyR	Cell based expression of GlyRIalpha	Progressive encephalomyelitis with rigidity and myoclonus, or stiff person syndrome or hyperekplexia	Probably very rare Immunotherapy responsive
Glutamic acid decarboxylase	Various assays employing recombinant GAD	Increasing recognition of the usefulness of GAD antibodies as a marker for stiff person syndrome, autoimmune cerebellar ataxia or limbic encephalitis	Variably immunotherapy responsive but treatments may need to be started early

GABAR = gamma-amino butyric acid receptor

disturbance.<sup>6,7</sup> In fact, although the antibodies are still measured by the radioimmunoprecipitation assay (see Table), it has become clear that most of the antibodies in the patients with CNS diseases are not directed against the VGKCs themselves but against associated proteins that are part of the VGKC-complexes found in the CNS and PNS. This new knowledge will begin to provide an explanation for the differing clinical phenotypes associated with "VGKC" antibodies (Irani and Vincent in preparation).

The next major development concerned a completely different sort of channel antibody. Aquaporin4 (AQP4) is a water channel and the target for antibodies in neuromyelitis optica.<sup>8</sup> Measurement of the antibodies is best determined using a cell-based approach (as used for several assays, see Figure), although there are now commercial ELISAs and other assays appearing. It is proving to be very helpful in distinguishing these patients from typical MS, and by so doing this ensures that they receive appropriate treatments. The antibodies are also found in

### Cell-based assays for the autoimmune channelopathies



Cell based assays are proving to be the best method for the detection of antibodies to membrane proteins. The antigen (pale blue) is expressed on the surface of a cell transfected with cDNAs encoding the antigen subunits. The patient's antibodies (yellow) are detected by binding of a fluorescent anti-human IgG (red). The antigens can be tagged with another fluorescent colour if required (eg. green, not shown), and sensitivity can be increased by clustering the antigen using naturally occurring intracellular scaffold proteins –eg. RAPSyn (dark green) that clusters AChRs at the neuromuscular junction.

## Collectively these (CNS) syndromes are now proving to be some of the most satisfying diseases to encounter, and our laboratory is detecting one or other of these antibodies in around up to 20 patients each week

children with this condition.<sup>9</sup> The antibodies are complement fixing and damage astrocytes directly, and probably neurons and oligodendrocytes indirectly.<sup>10</sup> There are recent reports of passive transfer models that demonstrate the pathogenicity of the antibodies.

Also exciting has been the discovery of NMDAR antibodies in patients with a complex neurological syndrome often associated with ovarian teratomas in young women.<sup>11</sup> The antibodies were first detected by binding to the neuropil of the hippocampus in rat brain sections – a technique which illustrates the presence of a highly specific antibody but does not identify the target. Hippocampal neuronal cultures showed that the antibodies were directed towards cell-surface determinants and were likely to be pathogenic (in comparison with many of the antibodies detected by binding to brain tissue sections, such as anti-Hu, or anti-Yo, which are directed towards intracellular antigens and merely markers for a paraneoplastic disease process rather than pathogenic<sup>12</sup>). The target was identified as the NMDAR. Whereas at first the antibodies were thought to be predominantly against the NR2B subunit, they are now known to be mainly against the NR1 subunit. They are best identified by binding to the surface of cells expressing the NR1/NR2B subunit complex and are now being found in many patients with an acute onset of neurological distur-

bance that includes combinations of amnesia, seizures, personality change or frank psychosis, with movement disorders, autonomic disturbance and brain stem signs. Curiously, despite the very dramatic and disturbing clinical picture, the MRIs can be normal or non-specific. Now it is recognized that NMDAR antibodies can be present without tumours in both sexes (Irani et al submitted) and in children.<sup>13</sup> We found the same antibodies in children who had been given a diagnosis of dyskinetic encephalitis lethargica<sup>14</sup> – a rather satisfying development in the understanding of this historic disease of the early 20th century.

Three other antibodies to look out for in the future are AMPAR or GABA(B)R in limbic encephalitis and GlyRs in stiff person syndrome and its variations. Each of these antibodies has been recently identified by one group only, and their frequency is not yet clear. Nevertheless, since the syndromes associated with these antibodies appear to be immunotherapy responsive, and may be associated with tumours (AMPA, GABA(B)R) their use in routine screening is likely to be taken up.

Finally, antibodies to GAD have always been a bit confusing. GAD is an intracellular enzyme and antibodies to it should not be pathogenic - unless they can get into the cell, or unless GAD is also expressed on the cell surface, neither of which have been convinc-

ingly demonstrated. Nevertheless, GAD antibodies at very high titres are being identified in patients not only with stiff person syndrome or autoimmune cerebellar ataxia but also in a few epilepsy patients and now in limbic encephalitis.<sup>15</sup> Unfortunately, the GAD antibodies are seldom sought early on in the disease course, and treatments are not often effective, but it seems highly likely that GAD antibodies are a marker for an immune-mediated process that might respond well to treatments if started early enough.

These brief comments highlight the marked change in direction that antibody-mediated conditions are undergoing and the importance of being aware that CNS conditions can be antibody-mediated and treatment responsive.<sup>16,17</sup> Although individually not very common, collectively these syndromes are now proving to be some of the most satisfying diseases to encounter, and our laboratory is detecting one or other of these antibodies in around up to 20 patients each week. All that is required now is for companies to take up this area enthusiastically in order to provide the most sensitive assays. The cell-based approach is being used for most of the membrane receptor antigens (see Table and Figure) and is not easy to provide in a suitable form for routine diagnostic testing, but this is a challenge that must be overcome. ♦

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